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Entyloma scandicis, a new smut fungus on *Scandix verna* from Mediterranean forests of Israel

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Abstract — The morphology and phylogeny of a species of *Entyloma* on *Scandix verna* (*Apiaceae*, *Apioideae, Scandiceae*) collected in the Mount Carmel and Lower Galilee regions of Israel were studied using light microscopy and ITS rDNA sequence analyses. The fungus differs morphologically from other species on hosts of subfamily *Apioideae: E. bupleuri, E. helosciadii* s.l., *E. kundmanniae, E. magocsyanum,* and *E. pastinacae*. Molecular phylogenetic analyses revealed that all specimens of *Entyloma* from *Scandix* represent a monophyletic lineage, sister to *E. magocsyanum*. As a result, the smut in leaves of *Scandix verna* is described and illustrated here as a new species, *Entyloma scandicis*.

Key words — *Entylomatales*, evolution, plant pathogens

Introduction

Entyloma contains more than 170 species of dicotyledon-parasitizing smut fungi, which are found on all continents except Antarctica (Vánky 2012). The phylogenetic relationships of *Entyloma* species based on ITS rDNA were established by Begerow et al. (2002), who showed that *Entyloma* evolution has been driven by its hosts. In a more recent study, both ITS sequence analysis and morphological data were used to delimit *Entyloma* spp. on *Eryngium* (Savchenko et al. 2014). The integration of molecular phylogenetic analyses, host plant taxonomy, and morphology provides a natural classification of these fungi.

Table 1. GenBank sequences used in this study

Species	Genbank No.	Reference
Entyloma atlanticum	AY081018	Begerow et al., 2002
E. arnicale	AY854965	Boekhout et al., 2006
E. australe	AY081019	Begerow et al., 2002
E. bidentis	AY081020	Begerow et al., 2002
E. browalliae	AY081021	Begerow et al., 2002
E. calceolariae	AY081022	Begerow et al., 2002
E. calendulae	AY081023	Begerow et al., 2002
E. carmeli	KF310892, KF310895	Savchenko et al., 2014
E. chrysosplenii	AY081024	Begerow et al., 2002
E. comaclinii	AY081025	Begerow et al., 2002
E. compositarum	AY854966	Boekhout et al., 2006
E. corydalis	AY081027	Begerow et al., 2002
E. costaricense	AY081028	Begerow et al., 2002
E. doebbeleri	AY081032	Begerow et al., 2002
E. eryngii	AY854972, AY081033, KF310896, KF310897	Begerow et al., 2002; Boekhout et al., 2006; Savchenko et al., 2014
E. eryngii-cretici	KF310894, KF310895	Savchenko et al., 2014
E. eryngii-plani	AY081034	Begerow et al., 2002
E. eschscholziae	KC456226	Genbank, unpublished
E. fergussonii	AY854971	Boekhout et al., 2006
E. ficariae	AY081035, JQ586199	Begerow et al., 2002; Savchenko et al. 2012
E. fuscum	AY081036	Begerow et al., 2002
E. gaillardianum	AY081037	Begerow et al., 2002
E. guaraniticum	AY081038	Begerow et al., 2002
E. hieracii	AY081039	Begerow et al., 2002
E. holwayi	AY081040	Begerow et al., 2002
E. linariae	AY081041	Begerow et al., 2002
E. lobeliae	AY081042	Begerow et al., 2002
E. madiae	AY081043	Begerow et al., 2002
E. magocsyanum	KF310891	Savchenko et al., 2014
E. matricariae	AY081044	Begerow et al., 2002
E. microsporum	AY081045	Begerow et al., 2002
E. polysporum	AY081046	Begerow et al., 2002
E. serotinum	AY081048	Begerow et al., 2002
E. ranunculi-repentis	AY081047	Begerow et al., 2002

Apiaceae harbors ca. twenty *Entyloma* species (Begerow et al. 2002; Vánky 2012), five of which are known on hosts of subfamily *Apioideae: E. bupleuri* Lindr. on *Bupleurum* (Liro 1904), *E. helosciadii* Magnus on *Helosciadium* (Magnus 1882), *E. kundmanniae* Malençon & Massenot on *Kundmannia* (Guyot et al. 1969), *E. magocsyanum* Bubák on *Tordylium* (Bubák 1907), and *E. pastinacae* Jaap on *Pastinaca* (Jaap 1916). Both *Entyloma flavum* Cif. on *Berula* and *Sium* (Ciferri 1924) and *E. oenanthes* Maire on *Oenanthe* (Maire et al. 1901) are considered conspecific with *E. helosciadii* s.l. (Reid 1957; Vánky 1994). Species delimitation of *Entyloma* on *Apioideae* is based primarily on host plant taxonomy and secondarily on minor differences in sori and spore morphologies. The analysis by Savchenko et al. (2014) clustered together *Entyloma* species on *Apiaceae*. That analysis was based mostly on the smut parasites of *Eryngium* (subfamily *Saniculoideae*) and included only one species (*Entyloma magocsyanum*) that parasitized a host from subfamily *Apioideae*.

During a 2011 survey on the diversity of smut fungi in Israel, a few infected *Scandix verna* plants were collected in two localities in northern Israel. Previously, no *Entyloma* species had been recorded on *Scandix*. The present study aims to resolve the specific status of the smut on *S. verna* through morphological and ITS sequence analyses and to determine its phylogenetic affinities within *Entyloma*.

Materials & methods

Herbarium samples are deposited in Haifa University Herbarium, Haifa, Israel (HAI); Washington State University Herbarium, Pullman, U.S.A. (WSP); and United States National Fungus Collection, Beltsville, U.S.A. (BPI). We were not able to include an *Entyloma kundmanniae* specimen in our analysis, so the information given on that species is based on Guyot et al. (1969) and Vánky (2012). Our hologenetype concept follows Chakrabarty (2010).

Sorus and spore characteristics were studied using dried herbarium material. Specimens were examined by light microscopy (LM). Pictures of sori were taken with a Canon Power Shot G10 camera. For LM, small pieces of leaf tissue with sori were mounted in 90% lactic acid on a microscope slide to which several drops of distilled water were added after which the slide was heated to the boiling point and cooled. The softened sori were cut in long pieces and squashed with a lancet, covered with a cover glass, gently heated to boiling point to eliminate air bubbles, and then examined under a Carl Zeiss Axiostar™ light microscope at 1000× magnification. LM photographs were taken with a Canon Power Shot G10 camera. At least 50 spores were measured from each collection, and the variation is presented as a range, with extreme values given in parentheses. Mean and standard deviations (SD) calculated from spores measured in all specimens are provided after the spore size ranges.

For SEM studies, spores were attached to metal stubs by double-sided adhesive tape and coated with gold. Spore surface ornamentation was observed at 15 kV and

photographed with a JEOL JSM-6700F scanning electron microscope with a working distance of ca. 12–13 mm.

Most sequences were obtained from GenBank (Table 1). Three additional sequences were generated for this analysis (Table 2). Genomic DNA was isolated from sori removed from herbarium specimens and lysed in 1.5 mL tubes for 1 min using FastPrep®24. Tubes were incubated in a water bath for 5 hours at 55 °C, and DNA extracted using DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's instructions.

All amplifications were performed in 20 µl aliquots on an Applied Biosystems® GeneAmp 9700 thermal cycler. ITS5 or ITS1 were used as the forward primer and ITS4 as the reverse primer (White et al. 1990).

Standard cycling parameters with an annealing temperature of 57 °C were used for amplification. PCR products were purified with USB ExoSAP-IT according to the manufacturer's instructions and amplified with respective forward and reverse PCR primers with the BigDye® Terminator v3.1 Cycle Sequencing Kit. Those products were sequenced on an ABI PRISM® 3100 Genetic Analyzer.

The data were initially assembled and edited in Sequencher 4.5 for Windows, and the consensus sequences were aligned using MAFFTv6 (Katoh et al. 2002; Katoh & Toh 2008) under the default settings. Ambiguously aligned regions were recorded using GBlocks 091.b (Castresana 2000) with these options: minimum number of sequences for a conserved position to 29, minimum number of sequences for a flank position to 29, maximum number of contiguous non-conserved positions to 8, minimum length of a block to 5 and allowed gap positions to with half, and excluded from the analyses.

PAUPv40b10 was used for parsimony analysis, with trees inferred using the heuristic search option with 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed, and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC], and Homoplasy Index [HI] were calculated for trees generated under different optimality criteria. Bootstrap analysis (BS) (Hill & Bull 1993) was based on 1000 replications.

Bayesian analysis using a Monte Carlo Markov Chain (MCMC) technique was implemented using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Four MCMC chains were run simultaneously, starting from random trees, for 1,000,000 generations. Trees were sampled every 100th generation for a total of 10,000 trees. The first 2000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (PP) (Rannala & Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 8,000 trees. This analysis was done four times starting from different random trees to ensure that trees from the same tree space were being sampled during each analysis.

Trees were rooted using *Entyloma atlanticum* as in Begerow et al. (2002).

Results

Morphological analyses

All examined *Entyloma* specimens (Table 2) produced sori in the leaves of their hosts. The color of sori ranged from white in young sori to pale yellowish

Table 2. *Entyloma* specimens examined in this study

Species	Host	Voucher	Origin	Collector/ Year	GenBank
E. bupleuri	*Bupleurum subovatum*	HUJ	Israel	Chabelska/1942	
E. helosciadii	*Apium nodiflorum*	BPI 175486	Ireland	O'Connor/1945	
	Apium nodiflorum*	BPI 175487	Algeria	Maire/1916	
	Berula angustifolia	WSP 34402	Germany	Unknown/1900	
	Helosciadium repens	WSP 34403	Algeria	Unknown/1904	
	Oenanthe silaifolia	WSP 70797	Romania	Vánky1965	
E. magocsyanum	*Tordylium cordatum*	HAI 4625	Israel	Savchenko/2011	KF310891
	Tordylium maximum	WSP 66423	Romania	Vánky/1966	
	Tordylium maximum	BPI 175835	Romania	Bubák/1905	
	Tordylium maximum	BPI 175836	Romania	Bubák/1905	
E. pastinacae	*Pastinaca opaca*	BPI 176163	Romania	Săvulescu & Rayss/1930	
E. scandicis	*Scandix verna*	HAI 4799	Israel	Savchenko/2011	KF447773
	Scandix verna	HAI 4805	Israel	Savchenko/2011	KF447774
	Scandix verna	HAI 4806	Israel	Savchenko/2011	KF447775

*as *Helosciadium*

brown in old sori. The spores in all specimens were solitary, in chains, or in groups (but never crowded) and variable in size within a collection. Asexual states were present in all species except *E. bupleuri.* Spore sizes were similar in all species, but spore wall thicknesses varied from 0.5–1 μm in *Entyloma magocsyanum* to 1–3 μm in *E. helosciadii.* The color of sori varied from brown in *Entyloma bupleuri* to white in *E. scandicis* (and sometimes *E. helosciadii*).

Phylogenetic analyses

The ITS alignment included 48 sequences (including three sequences of *Entyloma* from *Scandix verna*) comprising 655 characters (including gaps). Parsimony analysis revealed that 477 characters are constant and of the variable characters 77 are parsimony-uninformative and 101 are parsimony informative. The parsimony analysis yielded 45 equally parsimonious trees, and the strict consensus tree of all equally parsimonious trees was used.

The different runs of Bayesian phylogenetic analyses yielded consistent topologies. We present the consensus tree of one run of Bayesian phylogenetic analyses to illustrate our results (Fig. 1). All analyses clustered together (with high support: PP = 1.0; BS = 93) the sequences of the smut on *Scandix verna*, which formed the sister lineage to *Entyloma magocsyanum.* The sequences of the *Scandix verna* smut from Carmel and Lower Galilee regions were identical to one another.

The phylogenetic relationships among species of *Entyloma* on *Apiaceae* inferred here are congruent with those in the study on *Entyloma* on *Eryngium* (Savchenko et al. 2014).

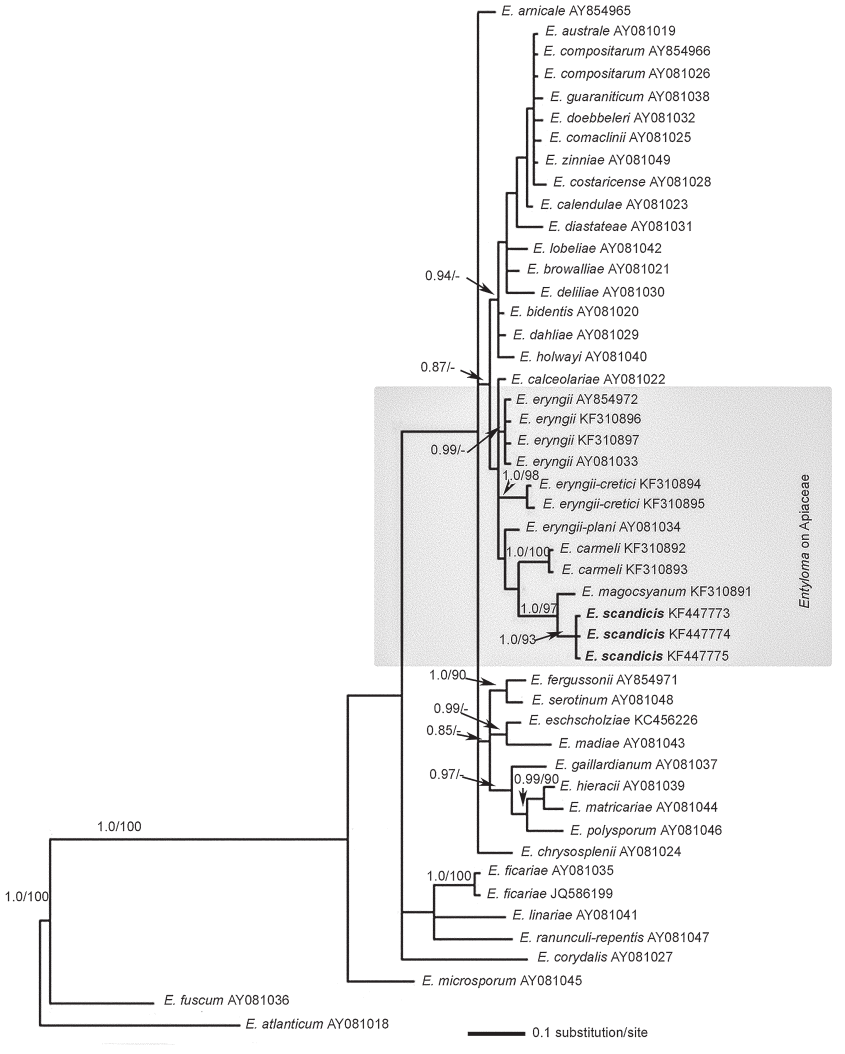

Fig. 1 Bayesian inference of phylogenetic relationships of *Entyloma* spp. resulting from the analysis of ITS nucleotide sequence data. Numbers on branches are estimates for PPs >0.8 from Bayesian inference (before /) and BS values >70 (after /). The tree was rooted using *Entyloma atlanticum*.

Taxonomy

Entyloma scandicis K.G. Savchenko, Carris, Castl., Heluta, Wasser & Nevo **sp. nov.** FIGURE 2

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Differs from *Entyloma helosciadii* by its paler spores and its specialization on *Scandix*.

TYPE: Israel. Lower Galilee, 2 km E of Kiryat Ata, 32°82′26″N 35°13′90″E, ca. 350 m asl, on *Scandix verna* O. Cohen, 15.04.2011, leg. K.G. Savchenko (**Holotype**, HAI 4799: GenBank KF447773).

ETYMOLOGY: The name of the species refers to the host plant genus, *Scandix*.

Sori in leaves, in round or elongate to polyangular amphigenous flat scattered or sometimes gregarious spots, initially white, later pale yellowish brown, 0.2–1 mm diameter or larger when confluent. Spores singular or in small irregular groups, sometimes in chains, globose, subglobose, ovoid, ellipsoidal to slightly irregular, (7) 8–11 × 9.5–15 (17) µm diameter [av. ± SD, 9.8 ± 1.6 × 12.6 ± 2.5 µm], subhyaline. Spore wall uniform, 1.5–2 (2.5) µm thick, hyaline to subhyaline, smooth. Anamorphic state present as fascicles of conidia and conidiophores, forming the amphigenous, white cover.

ADDITIONAL SPECIMENS EXAMINED: **ISRAEL**, **HAIFA**, Mount Carmel, University of Haifa campus, 32°75′83″N 35°02′32″E, ca. 430 m a.s.l., on *Scandix verna*, 11.04.2011, leg. K.G. Savchenko (HAI 4805; GenBank KF447774); Mount Carmel, Carmel National Park, 32°75′86″N 35°02′41″E, ca. 440 m asl, on *Scandix verna*, 30.03.2011, leg. K.G. Savchenko (HAI 4806; GenBank KF447775).

COMMENTS – The infected plants were found in March and April in two localities in northern Israel in different habitats. At Mount Carmel, *Entyloma scandicis* occurred on host plants in a shaded forest community under *Pinus halepensis*. In the Lower Galilee location, the fungus occurred on host plants in an open forest in glades between *Cupressus sempervirens*. Both locations are typical for Israel's Mediterranean floristic zone (Zohary 1973). Certainly, *Entyloma scandicis* is a Mediterranean forest species, hitherto not known outside of northern Israel. *Entyloma scandicis* was not found in steppe and semi-desert areas within the area of host distribution despite examination of thousands of host plants.

Discussion

Scandix is a monophyletic genus of the tribe *Scandiceae* (Downie et al. 1998, 2000; Lee & Downie 2000; Spalik et al. 2001) of which no members were previously known to host smut fungi (Vánky 2012). Our molecular phylogenetic analyses and morphological data have helped to resolve the systematic position of *Entyloma* on *Scandix verna*.

Entyloma scandicis can be distinguished from *E. bupleuri* by the sorus color (yellowish white in *E. scandicis,* brown in *E. bupleuri*) and the presence of an *Entylomella*-like asexual stage in *E. scandicis.* The new species can be separated from *Entyloma kundmanniae, E. magocsyanum,* and *E. pastinacae* by thicker spore walls (1.5–2.5 µm in *E. scandicis* vs. ≤1.5 µm in the other three). In several aspects, such as in spore dimensions, *Entyloma scandicis* resembles *E. helosciadii*, which differs in its pale to dark yellow spores.

ITS phylogenetic analysis recovered all three *Entyloma* specimens on *Scandix verna* in a strongly supported monophyletic group forming an independent lineage (Fig. 1). Morphologically there is no difference between the Mt Carmel and Lower Galilee specimens. As in the molecular phylogenetic analysis of a smaller sampling of *Entyloma* species on *Apiaceae* (Savchenko et al. 2014), our analysis clustered together all *Entyloma* species on *Apiaceae* within the /apiaceae clade and in sharing a common ancestor with other *Entyloma* groups on asterid hosts. Interestingly, *E. calceolariae* Lagerh., a parasite on species in the family *Calceolariaceae*, also clustered within the /apiaceae clade (Fig. 1).

So far, only two species of *Entyloma* on *Apioideae—E. magocsyanum* and *E. scandicis*—have undergone molecular phylogenetic analyses (Savchenko et al. 2014). It should be noted that molecular-oriented studies have implied significant radiation for some *Entyloma* species (Savchenko et al. 2014; Vánky & Lutz 2010), which supports the hypothesis that *Entyloma* species have relatively narrow host ranges restricted to a single genus or, in some cases, a single host species (Savchenko et al. 2014; Savile 1947; Vánky 1994, 2009). Additional sequencing is needed to resolve the taxonomy of *Entyloma helosciadii,* a complex of three species known to infect hosts from four different genera in the tribe *Oenantheae* (Reid 1957; Vánky 1994). Another taxonomic problem is the possible conspecificity of the pathogenic *Entyloma magocsyanum* and *E. pastinacae*, both with hosts in tribe *Tordyleae* and with no diagnostic morphological differences.

This study shows that genetic divergence, a different host tribe, and different spore and soral morphologies support *Entyloma scandicis* on *Scandix verna* as a new species. This species fills a gap in the distribution of *Entyloma* on representatives of the tribe *Scandiceae*. Future molecular phylogenetic studies combined with morphological methods may well reveal higher diversity within the species of *Entyloma* on *Apioideae*.

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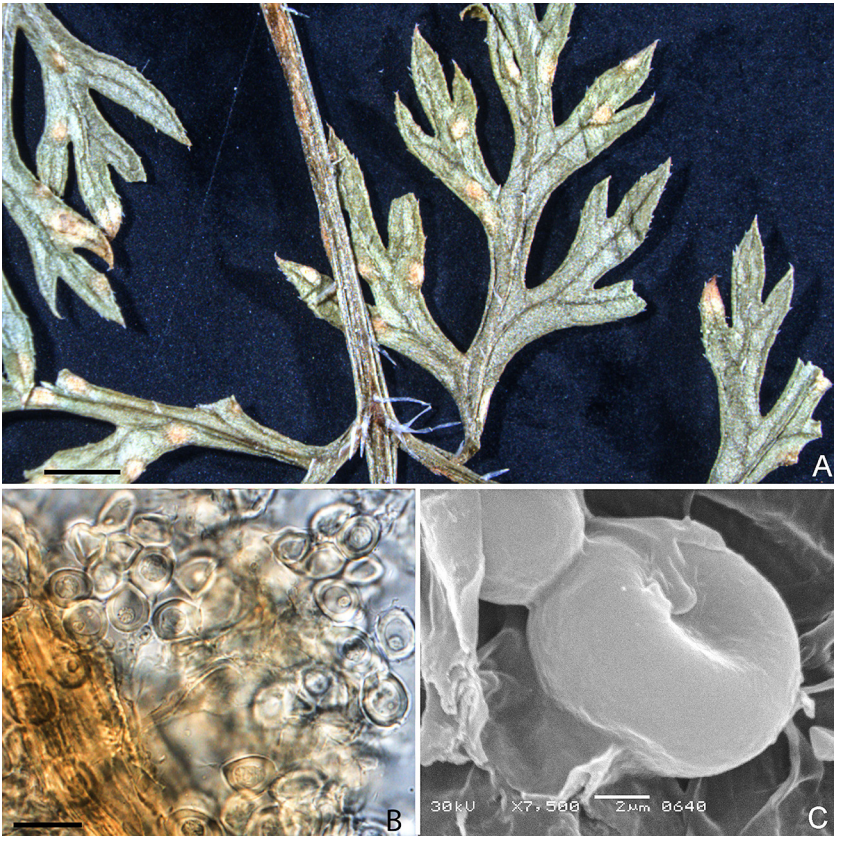

Fig. 2 *Entyloma scandicis* (holotype, HAI 4799): A. sori in leaves of *Scandix verna*; B. spores (LM); C. spores (SEM). Scale bars: A = 2 mm, B = 20 µm, C = 2 µm.

Literature cited

Begerow D, Lutz M, Oberwinkler F. 2002. Implications of molecular characters for the phylogeny of the genus *Entyloma*. Mycological Research 106: 1392–1399. http://dx.doi.org/10.1017/S0953756202006962

Boekhout T, Gildemacher P, Theelen B, Müller WH, Heijne B, Lutz M. 2006. Extensive colonization of apples by smut anamorphs causes a new postharvest disorder. FEMS Yeast Research 6: 63–76. http://dx.doi.org/10.1111/j.1567-1364.2005.00002.x

Bubák F. 1907. Ein Beitrag zur Pilzflora von Ungarn. Növénite Közlem 6: 19–56.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540–552. http://dx.doi.org/10.1093/oxfordjournals.molbev.a026334

Chakrabarty P. 2010. Genetypes: a concept to help integrate molecular phylogenetics and taxonomy. Zootaxa 2632: 67–68.

Ciferri R. 1924. Prima contribuzione allo studio degli Ustilaginales (No. 1-22). Bolletino della Societa Botanica Italiana 1924: 46–59.

Downie S, Ramanath S, Katz-Downie DS, Llanas E. 1998. Molecular systematics of *Apiaceae* subfamily *Apioideae*: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid RPO C1 intron sequences. American Journal of Botany 85: 563–591. http://dx.doi.org/10.2307/2446441

Downie SR, Katz-Downie DS, Watson MF. 2000. A phylogeny of the flowering plant family *Apiaceae* based on chloroplast DNA rpl16 and rpoC1 intron sequences: towards a suprageneric classification of subfamily *Apioideae*. American Journal of Botany 87: 273–292. http://dx.doi.org/10.2307/2656915

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 817: 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754

Guyot L, Malençon G, Massenot M. 1969. Quatrième contribution à l'étude des *Ustilaginales* parasites du Bassin méditerranéen occidental (Afrique du Nord, Espaqne, Italie). Revue de Mycologie 34: 192–219.

Jaap O. 1916. Beiträge zur Kenntnis der Pilze Dalmatiens. Annales Mycologici 14: 1–44.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program (outlines version 6). Briefs in Bioinformatics 9: 286–298. http://dx.doi.org/10.1093/bib/bbn013

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acid Research 30: 3059–3066. http://dx.doi.org/10.1093/nar/gkf436

Lee BY, Downie SR. 2000. Phylogenetic analysis of cpDNA restriction sites and *rps*16 intron sequences reveals relationships among *Apiaceae* tribes *Caucalideae*, *Scandiceae* and related taxa. Plant Systematics and Evolution 221: 35–60. http://dx.doi.org/10.1007/BF01086379

Liro JI. 1904. Mykologische Mitteilungen 11-15. Acta Societatis pro Fauna et Flora Fennica 26: 1–18.

Magnus P. 1882. Ein neues *Entyloma* auf *Helosciadium nodiflorum* K. Hedwigia 21: 129–130.

Maire R, Dumée P, Lutz L. 1901. Prodrome d'une flore mycologique de la Corse. Bulletin de la Société botanique de France 48: 179–247.

Rannala B, Yang Z. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. Journal of Molecular Evolution 43: 304–311. http://dx.doi.org/10.1007/BF02338839

Reid DA. 1957. New or interesting records of British *Ustilaginales*. Transactions of the British Mycological Society 40: 193–199. http://dx.doi.org/10.1016/S0007-1536(57)80003-5

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Savchenko KG, Heluta VP, Hirylovich IS, Wasser SP, Nevo E. 2012. Notes on some Eurasian species of *Anthracoidea* and *Entyloma*. Mycotaxon 121: 53–62. http://dx.doi.org/10.5248/121.53

Savchenko KG, Carris LM, Castlebury LA, Heluta VP, Wasser SP, Nevo E. 2014. Revision of *Entyloma* (*Entylomatales*, *Exobasidiomycetes*) on *Eryngium*. Mycologia 106: 797–810. http://dx.doi.org/10.3852/13-317

Savile DBO. 1947. A study of the species of *Entyloma* on North American composites. Canadian Journal of Research, Section C, Botanical Sciences 25: 105–120. http://dx.doi.org/10.1139/cjr47c-011

Spalik K, Wojewódzka A, Downie SR. 2001. Delimitation of genera in *Apiaceae* within examples from *Scandiceae* subtribe *Scandicinae*. Edinburgh Journal of Botany 58: 331–346. http://dx.doi.org/10.1017/S096042860100066X

Vánky K. 1994. European smut fungi. Stuttgart, Germany: Gustav Fischer Verlag.

Vánky K. 2009. Taxonomic studies on *Ustilaginomycetes* – 29. Mycotaxon 110: 289–324. http://dx.doi.org/10.5248/110.289

Vánky K. 2012. Smut fungi of the world. St. Paul, APS Press.

Vánky K, Lutz M. 2010. *Entyloma majewskii* sp. nov. (*Entylomataceae*) on *Ranunculus ficaria* from Iran. Polish Botanical Journal 55: 271–279.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA sequences for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR protocols: a guide to methods and applications. San Diego, Academic Press.

Zohary M. 1973. Geobotanical foundation of the Middle East. Gustav Fischer Verlag, Stuttgart.